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(54) Title: HYDROXY AND ETHER-CONTAINING OXYALKYLENE ESTERS AND USES THEREOF			
(57) Abstract			
<p>This invention relates to compositions for and methods of treating, preventing or ameliorating cancer and other proliferative diseases as well as methods of inducing wound healing, treating cutaneous ulcers, treating gastrointestinal disorders, treating blood disorders such as anemias, immunomodulation, enhancing recombinant gene expression, treating insulin-dependent patients, treating cystic fibrosis patients, inhibiting telomerase activity, treating virus-associated tumors, especially EBV-associated tumors, augmenting expression of tumor suppressor genes, inducing tolerance to antigens, or treating, preventing or ameliorating protozoan infection or inhibiting histone deacetylase in cells. The compositions of the invention are to and the methods of the invention use hydroxy and ether-containing oxyalkylene esters.</p>			

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HYDROXY AND ETHER-CONTAINING  
OXYALKYLENE ESTERS AND USES THEREOF5 FIELD OF THE INVENTION

This invention relates to compositions for and methods of treating, preventing or ameliorating cancer and other proliferative diseases as well as methods of inducing wound healing, treating cutaneous ulcers, 10 treating gastrointestinal disorders, treating blood disorders such as anemias, immunomodulation, enhancing recombinant gene expression, treating insulin-dependent patients, treating cystic fibrosis patients, inhibiting telomerase activity, treating virus-associated tumors, 15 especially EBV-associated tumors, modulating gene expression and particularly augmenting expression of tumor suppressor genes, inducing tolerance to antigens or treating, preventing or ameliorating protozoan infection or inhibiting histone deacetylase in cells. The 20 compositions of the invention are to and the methods of the invention use hydroxy and ether-containing oxyalkylene esters.

25 BACKGROUND OF THE INVENTION

Butyric acid (BA) is a natural product. It is supplied to mammals from two main sources: 1) the diet, mainly from dairy fat, and 2) from the bacterial fermentation of unabsorbed carbohydrates in the colon, where it reaches mM concentrations (Cummings, Gut 22:763-779, 1982; Leder et al., Cell 5:319-322, 1975).

BA has been known for nearly the last three decades to be a potent differentiating and antiproliferative agent in a wide spectra of neoplastic cells *in vitro* (Prasad, Life Sci. 27:1351-1358, 1980). In cancer cells, 30 BA has been reported to induce cellular and biochemical changes, e.g., in cell morphology, enzyme activity, receptor expression and cell-surface antigens (Nordenberg et al., Exp. Cell Res. 162:77-85, 1986; Nordenberg et 35

al., Br. J. Cancer 56:493-497, 1987; and Fishman et al., J. Biol. Chem. 254:4342-4344, 1979).

5        Although BA or its sodium salt (sodium butyrate, SB) has been the subject of numerous studies, its mode of action is unclear. The most specific effect of butyric acid is inhibition of nuclear deacetylase(s), resulting in hyperacetylation of histones H3 and H4 (Riggs, et al., Nature 263:462-464, 1977). Increased histone acetylation, following treatment with BA has been correlated with changes in transcriptional activity and the differentiated state of cells (Thorne et al., Eur. J. Biochem. 193:701-713, 1990). BA also exerts other nuclear actions, including modifications in the extent of phosphorylation (Boffa et al., J. Biol. Chem. 256:9612-15 9621, 1981) and methylation (Haan et al., Cancer Res. 46:713-716, 1986). Other cellular organelles, e.g., cytoskeleton and membrane composition and function, have been shown to be affected by BA (Bourgeade et al., J. Interferon Res. 1:323-332, 1981). Modulations in the expression of oncogenes and suppressor genes by BA were demonstrated in several cell types. Toscani et al., reported alterations in c-myc, p53 thymidine kinase, c-fos and AP2 in 3T3 fibroblasts (Oncogene Res. 3:223-238, 1988). A decrease in the expression of c-myc and H-ras 20 oncogenes in B16 melanoma and in c-myc in HL-60 promyelocytic leukemia was also reported (Prasad et al., Biochem. Cell Biol. 68:1250-1255, 1992; and Rabizadeh et al., FEBS Lett. 328:225-229, 1993).

25        BA has been reported to induce apoptosis, i.e., programmed cell death. SB has been shown to produce apoptosis *in vitro* in human colon carcinoma, leukemia and retinoblastoma cell lines (Bhatia et al., Cell Growth Diff. 6:937-944, 1995; Conway et al., Oncol. Res. 7:289-297, 1993; Hague et al.; Int. J. Cancer 60:400-406, 30 35 1995). Apoptosis is the physiological mechanism for the elimination of cells in a controlled and timely manner.

Organisms maintain a delicate balance between cell proliferation and cell death, which when disrupted can tip the balance between cancer, in the case of over accumulation of cells, and degenerative diseases, in the 5 case of premature cell losses. Hence, inhibition of apoptosis can contribute to tumor growth and promote progression of neoplastic conditions.

The promising *in vitro* antitumor effects of BA and BA salts led to the initiation of clinical trials for the 10 treatment of cancer patients with observed minimal or transient efficacy [Novogrodsky et al., Cancer 51:9-14, 1983; Rephaeli et al., Intl. J. Oncol. 4:1387-1391, 1994; Miller et al., Eur. J. Cancer Clin. Oncol. 23:1283-1287, 1987].

15 Clinical trials have been conducted for the treatment of  $\beta$ -globin disorders (e.g.,  $\beta$ -thalassemia and sickle-cell anemia) using BA salts. The BA salts elevated expression of fetal hemoglobin (HbF), normally repressed in adults, and favorably modified the disease 20 symptoms in these patients (Stamatoyannopoulos et al., Ann. Rev. Med. 43:497-521, 1992). In this regard, arginine butyrate (AB) has been used in clinical trials with moderate efficacy (Perrine et al., N. Eng. J. Med. 328:81-86, 1993; Sher et al., N. Eng. J. Med. 332:1606-25 1610, 1995). The reported side effects of AB included hypokalemia, headache, nausea and vomiting in  $\beta$ -thalassemia and sickle-cell anemia patients.

30 Butyric acid derivatives with antitumor activity and immunomodulatory properties have been reported in U.S. Patent 5,200,553 and by Nudelman et al., 1992, J. Med. Chem. 35:687-694. The most active butyric acid prodrug reported in these references was pivaloyloxymethyl butyrate (AN-9). Similar compounds are reported for treating hemoglobinopathies (U.S. Patent No. 5,569,675).

35 BA and/or its analogues have also been reported to increase the expression of transfected DNA (Carstea et

al., 1993, Biophys. Biochem. Res. Comm. 192:649; Cheng et al., 1995, Am. J. Physical 268:L615-L624) and to induce tumor-restricted gene expression by adenovirus vectors (Tang et al., 1994, Cancer Gene Therapy 1:15-20).  
5 Tributyrin has been reported to enhance the expression of a reporter gene in primary and immortalized cell lines (Smith et al., 1995, Biotechniques 18:852-835).

10 However, BA and its salts are normally metabolized rapidly and have very short half-lives *in vivo*, thus the achievement and maintenance of effective plasma concentrations are problems associated with BA and BA salts, particularly for *in vivo* uses. BA and BA salts have required large doses to achieve even minimal therapeutic effects. Because of the high dosage, fluid 15 overload and mild alkalosis may occur. Patients receiving BA emanate an unpleasant odor that is socially unacceptable.

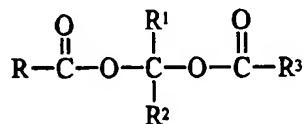
20 While BA salts have been shown to increase HbF expression, and appear to hold therapeutic promise with low toxicity in cancer patients, they nevertheless have shown low potency in *in vitro* assays and clinical trials. There remains a need to identify compounds as effective or more effective than BA or BA salts as differentiating or anti-proliferating agents for the treatment of 25 cancers. Such compounds need to have higher potency than BA without the problems associated with BA (such as bad odor). This need can be addressed by therapeutic compounds that either deliver BA to cells in a longer acting form or which have similar activity as BA but a 30 longer duration of effectiveness *in vivo*.

35 The compounds of this invention address these needs and are more potent than BA or BA salts for treating of cancers and other proliferative diseases, for treating gastrointestinal disorders, for wound healing, for treating blood disorders such as thalassemia, sickle cell anemia and other anemias, for modulating an immune

response, for enhancing recombinant gene expression, for treating insulin-dependent patients, for treating cystic fibrosis patients, for inhibiting telomerase activity, for treating virus-associated tumors, especially EBV-associated tumors, for modulating gene expression and particularly for augmenting expression of a tumor suppressor gene, for inducing tolerance to an antigen, for treating, preventing or ameliorating protozoan infection and for inhibiting histone deacetylase in cells. For example, one of the advantages of the compounds of the invention is increased hydrophilicity and thus easier administration, especially for intravenous administration.

15 SUMMARY OF THE INVENTION

The present invention is directed to compounds represented by the formula



wherein

20 R is C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl or C<sub>2</sub> to C<sub>10</sub> alkynyl, optionally substituted with hydroxy, alkoxy, halo, trifluoromethyl, aryl or heteroaryl;

25 R<sup>1</sup> and R<sup>2</sup> are independently H, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>2</sub>-C<sub>10</sub> alkenyl, or C<sub>2</sub>-C<sub>10</sub> alkynyl, any of which can be optionally substituted with halo, alkoxy, amino, trifluoromethyl, aryl or heteroaryl;

R<sup>3</sup> is

30 (1) C<sub>1</sub>-C<sub>12</sub> alkyl or C<sub>2</sub>-C<sub>10</sub> alkenyl substituted with from one to ten substituents selected from the group consisting of hydroxy, alkoxy, acyloxy, aroyloxy and aryloxy, and, optionally having one or more halo groups, wherein

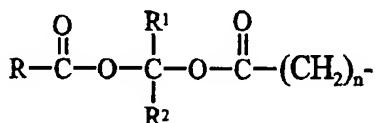
adjacent hydroxy groups, if present, can optionally be present as an acetonide,

(2)  $-(\text{CH}_2)_n-[\text{O}-(\text{CH}_2)_p]_m-\text{OR}^4$ ,

(3)  $-(\text{CH}_2)_n-\text{PEG-OR}^4$ , or

5 (4)  $-(\text{CH}_2)_n-\text{PPG-OR}^4$ ;

$\text{R}^4$  is  $\text{C}_1$  to  $\text{C}_6$  alkyl,  $\text{C}_2$  to  $\text{C}_6$  alkenyl, aryl, acyl, aroyl, alkoxy carbonyl, aminocarbonyl or



10 PEG is a polyethylene glycol moiety having an average molecular weight of 200-8000;

PPG is a polypropylene glycol moiety having an average molecular weight of 200-8000;

$\text{p}$  is 2 to 4;

$\text{n}$  is 1 to 5;

15  $\text{m}$  is 1 to 6; and

pharmaceutically-acceptable salts thereof;

with the proviso that when  $\text{R}$  is alkyl,  $\text{R}^3$  can not be monohydroxy- $\text{n}$ -propyl.

20 The above compounds are referred to herein as "Formula I compounds." In a preferred embodiment, the compounds of the invention are the compounds of Formula I, wherein  $\text{R}^3$  is  $-(\text{CH}_2)_n-[\text{O}-(\text{CH}_2)_p]_m-\text{OR}^4$ ,  $\text{p}$  is 2 or 3,  $\text{n}$  is 1 or 2 and  $\text{m}$  is 1, 2 or 3. In a more preferred embodiment the compounds of the invention are 2-(2-methoxyethoxy)acetyloxymethyl butyrate (Compound C), and 2-[2-(methoxyethoxy)ethoxy]acetyloxymethyl butyrate (Compound D), 1-[2-(2-methoxyethoxy)acetyl]oxyethyl butyrate and 1-{2-[2-(2-methoxyethoxy)ethoxy]acetyl}oxyethyl butyrate.

25 Another embodiment of the present invention is drawn to pharmaceutical compositions comprising a

therapeutically effective amount of a compound of Formula I and a pharmaceutically effective carrier or diluent.

A further embodiment of the present invention is directed to pharmaceutical compositions comprising a 5 therapeutically effective amount of a combination of a compound of Formula I with other anti-cancer or anti-neoplastic agents together with a pharmaceutically effective carrier or diluent.

Yet another embodiment of the present invention is 10 directed to a method of treating preventing or ameliorating cancer and other proliferative disorders using the compounds of Formula I. In a preferred embodiment, the compounds of the invention used in this and the other methods of the invention are the same 15 preferred and more preferred compounds of Formula I.

The compounds of Formula I are particularly useful 20 for methods of treating, preventing or ameliorating the effects of cancer and other proliferative disorders by acting as anti-proliferative or differentiating agents in subjects afflicted with such anomalies. Such disorders include but are not limited to leukemias, such as acute 25 promyelocytic leukemia, acute myeloid leukemia, and acute myelomonocytic leukemia; other myelodysplastic syndromes, multiple myeloma such as but not limited to breast carcinomas, cervical cancers, melanomas, colon cancers, nasopharyngeal carcinoma, non-Hodgkins lymphoma (NHL), 30 Kaposi's sarcoma, ovarian cancers, pancreatic cancers, hepatocarcinomas, prostate cancers, squamous carcinomas, other dermatologic malignancies, teratocarcinomas, T-cell lymphomas, lung tumors, gliomas, neuroblastomas, peripheral neuroectodermal tumors, rhabdomyosarcomas, and 35 prostate tumors and other solid tumors. It is also possible that compounds of Formula I have anti-proliferative effects on non-cancerous cells as well, and may be of use to treat benign tumors and other proliferative disorders such as psoriasis. Preferred is

the method for treating or ameliorating leukemia, squamous cell carcinoma and neuroblastoma.

5 The invention is further directed to a method of treating blood disorders by administering a therapeutically-effective amount of a compound of Formula I to a patient.

10 The blood disorders treatable in accordance with the invention include, but are not limited to, thalassemias, sickle cell anemias, infectious anemias, aplastic anemias, hypoplastic and hypoproliferative anemias, 15 sideroblastic anemias, myelophthisic anemias, antibody-mediated anemias, anemias due to chronic diseases and enzyme-deficiencies, and anemias due to blood loss, radiation therapy and chemotherapy. In this regard, these methods can include increasing hemoglobin content 20 in blood by administering a therapeutically-effective amount of a compound of Formula I to a subject.

Another embodiment of the invention is directed to a method of modulating an immune response in a host by 25 administering an amount of a compound of Formula I effective to modulate said immune response.

Modulation of the immune response includes enhancing cytokine secretion, inhibiting or delaying apoptosis in polymorphonuclear cells, enhancing polymorphonuclear cell 25 function by augmenting hematopoietic growth factor secretion, inducing expression of cell surface antigens in tumor cells, and enhancing progenitor cell recovery after bone marrow transplantation.

Another embodiment of the present invention is 30 directed to methods of treating, preventing or ameliorating cancer and other proliferative disorders by administering a therapeutically-effective amount of a compound of Formula I to a subject suffering from such disorders together with a pharmaceutical agent (e.g., a 35 known antiproliferative, differentiating or oncostatic agent) to thereby enhance the action of these agents.

The pharmaceutical agents of the invention for the above method include, but are not limited to, cytokines, interleukins, anti-cancer agents, chemotherapeutic agents, antibodies, conjugated antibodies, immune stimulants, antibiotics, hormone antagonists, and growth stimulants. The compounds of the invention can be administered prior to, after or concurrently with any of the agents.

Yet another embodiment of the invention is directed to a method of ameliorating the effects of a cytotoxic agent which comprises administering a therapeutically-effective amount of a cytotoxic agent with a compound of Formula I to a mammalian patient for a time and in an amount to induce growth arrest of rapidly-proliferating epithelial cells of the patient and thereby protect those cells from the cytotoxic effects of the agent. The cytotoxic agent can be a chemotherapeutic agent, an anticancer agent, or radiation therapy. Rapidly proliferating epithelial cells are found in hair follicles, the gastrointestinal tract and the bladder, for example. Such cells include hair follicle cells, or intestinal crypt cells. Rapidly proliferating cells are also found in the bone marrow and include bone marrow stem cells. In accordance with the invention the cytotoxic agent and the compound of Formula I can be administered simultaneously, or the cytotoxic agent can be administered prior to or after the compound of the invention. Administration (simultaneously or separately) can be done systemically or topically as determined by the indication. In addition, when the cytotoxic agent is radiation therapy, the compounds of the invention can be administered to a cancer patient pre- or post-radiation therapy to treat or ameliorate the effects of cancer.

A still further embodiment of the invention is directed to a method of inducing wound healing, treating cutaneous ulcers or treating a gastrointestinal disorder

by administering a therapeutically-effective amount of a compound of Formula I to a subject in need of such treatment. The cutaneous ulcers which can be treated in accordance with the methods of the invention include leg and decubitus ulcers, stasis ulcers, diabetic ulcers and atherosclerotic ulcers. With respect to wound healing, the compounds are useful in treating abrasions, incisions, burns, and other wounds. Gastrointestinal disorders treatable by the methods of the invention include colitis, inflammatory bowel disease, Crohn's disease and ulcerative colitis.

A further embodiment of the invention relates to a method of enhancing recombinant gene expression by treating a recombinant host cell containing an expression system for a mammalian gene product of interest with an expression-enhancing amount of a compound of Formula I, wherein said gene product is encoded by a butyric acid-responsive gene. The host cells can be mammalian cells, insect cells, yeast cells or bacterial cells and the correspondingly known expression systems for each of these host cells. The gene product can be any protein or peptide of interest, expression of which can be regulated or altered by butyric acid or a butyric acid salt. A butyric acid-responsive gene is a gene that has a promoter, enhancer element or other regulon that modulates expression of the gene under its control in response to butyric acid or a salt of butyric acid. For example, gene products contemplated for regulation in accordance with the invention include but are not limited to tumor suppressor genes (such as p53) and the  $\gamma$ -globin chain of fetal hemoglobin.

Yet a further embodiment of the invention is directed to a method of treating, preventing or ameliorating symptoms in insulin-dependent patients by administering an amount of a compound of Formula I effective to enhance insulin expression.

Yet another embodiment of the invention relates to a method of treating, preventing or ameliorating symptoms in cystic fibrosis patients by administering an amount of a compound of Formula I effective to enhance chloride channel expression.

Still another method of the invention is directed to a method of inhibiting telomerase activity in cancer cells by administering a telomerase-inhibiting amount of a compound of Formula I to the cells, wherein the amount is effective to decrease the telomerase activity of the cells and thereby inhibit the malignant progression of the cells. This method can be applied to *in vivo* or *in vitro* cells.

Another embodiment of this invention is directed to a method of treating, preventing or ameliorating virus-associated tumors by pre-, post or co-administering a therapeutically-effective amount of a compound of Formula I with a therapeutically-effective amount of an antiviral agent. Antiviral agents contemplated for use in the invention include ganciclovir, acyclovir and famciclovir, and preferably ganciclovir. The virus-associated tumors which can be treated, prevented or ameliorated in accordance with the invention include, but are not limited to, EBV-associated malignancy, Kaposi's sarcoma, AIDS-related lymphoma, hepatitis B-associated malignancy or hepatitis C associated malignancy. EBV-associated malignancies include nasopharyngeal carcinoma and non-Hodgkins' lymphoma and are preferred embodiments of the invention.

Further still the invention provides a method of modulating gene expression by treating a host or host cells with an amount of a compound of Formula I effective to enhance, augment or repress the expression of a gene of interest, preferably a butyric acid responsive gene. When expression of the gene of interest is to be enhanced or augmented, the gene can encode a gene product which is

or acts as a repressor of another gene, a tumor suppressor, an inducer of apoptosis or an inducer of differentiation. When expression of the gene of interest is to be repressed, the gene can encode a gene product which is or acts as an oncogene or an inhibitor of apoptosis. For example, the *bcl-2* gene encodes an inhibitor of apoptosis.

More particularly, the invention is directed to a method of augmenting gene expression, especially of a tumor suppressor gene, a butyric acid-responsive gene or a fetal hemoglobin gene, by treating a host or host cells with an expression-enhancing amount of a compound of Formula I. Preferably the host is a cancer patient. This method of the invention thus includes augmenting tumor suppressor gene expression in conjunction with *ex vivo* or *in vivo* gene therapy, i.e., the compound of the invention can be co-administered to the host during administration of gene therapy vectors or administration of the *ex vivo* transfected cells. Similarly, the compounds of the invention can be used to treat cells during the transfection step of *ex vivo* gene therapy. The hosts of the method therefore include cancer patients or other patients under going gene therapy. The host cells of the invention include hematopoietic cells such as stem cells and progenitor cells, e.g., or any other cell type used in *ex vivo* gene therapy.

Yet another embodiment of the invention is directed to a method of inducing tolerance to an antigen which comprises administering a therapeutically-effective amount of compound of Formula I. Preferably the antigen is a self-antigen. For example, the self antigen can be associated with an autoimmune disease, such as systemic lupus erythromatosus, rheumatoid arthritis, multiple sclerosis or diabetes. Alternatively, tolerance can be induced to one or more antigens present on a transplanted organ or cells.

Yet further the invention is directed to a method for treating, preventing, or ameliorating protozoan infection in a subject which comprises administering to said subject an effective amount of a compound of Formula I. The protozoan infections treatable in accordance with the invention include, but are not limited to, malaria, cryptosporidiosis, toxoplasmosis, or coccidiosis.

Still further the invention is directed to a method of inhibiting histone deacetylase in cells which comprises administering an effective amount of a compound of Formula I to said cells.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graphic illustration showing the *in vitro* inhibition of cellular growth (clonogenicity) by AB, Compound C and Compound D on established HL-60 and CFPAC cancer cell lines as a function of concentration (in mM).

Fig. 2 is a graphic illustration showing the *in vitro* inhibition of cellular growth by Compound C, compound D and AN9 on immortalized human keratinocytes as a function of concentration (in mM).

Fig. 3 is a graphic illustration showing the percentage of human leukemic cells (HL-60) expressing the phenotypic differentiation marker CD11b for cells which had been treated with Compound C or AN9 for 6 days. Concentrations are in  $\mu$ M.

#### DETAILED DESCRIPTION OF THE INVENTION

The compounds herein described may have asymmetric centers. All chiral, diastereomeric, and racemic forms are included in the present invention. Many geometric isomers of olefins and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention.

By "stable compound" or "stable structure" is meant herein a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

As used herein, "alkyl" means both branched- and straight-chain, saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. As used herein "lower alkyl" means an alkyl group having 1 to 5 carbon atoms. As used herein, "alkenyl" means hydrocarbon chains of either a straight or branched configuration and one or more unsaturated carbon-carbon bonds, such as ethenyl, propenyl, and the like. "Lower alkenyl" is an alkenyl group having 2 to 6 carbon atoms. As used herein, "alkynyl" means hydrocarbon chains of either a straight or branched configuration and one or more carbon-carbon triple bonds, such as ethynyl, propynyl and the like. "Lower alkynyl" is an alkynyl group having 2 to 6 carbon atoms. When the number of carbon atoms is not specified, then alkyl, alkenyl and alkynyl means lower alkyl, lower alkenyl and lower alkynyl, respectively.

As used herein, "aryl" includes "aryl" and "substituted aryl." Thus "aryl" of this invention means any stable 6- to 14-membered monocyclic, bicyclic or tricyclic ring, containing at least one aromatic carbon ring, for example, phenyl, naphthyl, indanyl, tetrahydronaphthyl (tetralinyl) and the like. The presence of substitution on the aryl group is optional, but when present, the substituents can be halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro, trifluoromethyl, acylamino or carbamoyl. When a substituted aryl group is present on R or R<sup>3</sup> in Formula I, then the aryl substituents do not include amino, acylamino or carbamoyl.

As used herein, the term "heteroaryl" includes "heteroaryl" and "substituted heteroaryl." Thus "heteroaryl" of this invention means a stable 5- to 10-

membered monocyclic or bicyclic heterocyclic ring which is aromatic, and which consists of carbon atoms and from 1 to 3 heteroatoms selected from the group consisting of N, O and S and wherein the nitrogen may optionally be 5 quaternized, and including any bicyclic group in which any of the above-defined heteroaryl rings is fused to a benzene ring. The heteroaryl ring may be attached to its pendant group at any heteroatom or carbon atom which results in a stable structure. The presence of 10 substitution on the heteroaryl group is optional and can be on a carbon atom, a nitrogen atom or other heteroatom if the resulting compound is stable and all the valencies of the atoms have been satisfied. When present, the 15 substituents of the substituted heteroaryl groups are the same as for the substituted aryl groups and also include alkylammonium salts when the substituent in an alkyl group attached to the nitrogen atom of the heteroaryl ring. These quaternized ammonium salts include halides, hydrohalides, sulfates, methosulfates, methanesulfonates, 20 toluenesulfonates, nitrates, phosphates, maleates, acetates, lactates or any other pharmaceutically acceptable salt. Examples of heteroaryl groups include, but are not limited to, pyridyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, 25 benzofuranyl, benzothienyl, indolyl, indolenyl, quinolinyl, isoquinolinyl and benzimidazolyl.

When a heteroaryl group is present on R in Formula I, then the heteroatoms can not be a nitrogen. Similarly, if this same heteroaryl group has any substituents, the 30 substituents do not include amino, acylamino or carbamoyl, i.e., there can not also be an amino-type nitrogen-containing substituent.

The term "substituted", as used herein, means that 35 one or more hydrogens on the designated atom are replaced with a selection from the indicated groups, provided that

the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound.

The substituents of the invention include, as indicated, halo, hydroxy, alkyl, alkoxy, amino, cyano, 5 nitro, trifluoromethyl, aryl, heteroaryl, acyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, acyloxy, aryloxy, acetonide and aroyl. These groups can be substituents for alkyl, alkenyl, alkynyl, aryl, and heteroaryl groups as indicated in accordance with the 10 invention. A "halo" group is a halogen, and includes fluoro, chloro, bromo and iodo groups. The term "alkoxy" refers to an alkyl group having at least one oxygen substituent represented by R-O-. The group "acyl" is represented by the formula R-C(O)- where R is alkyl. 15 "Arylcarbonyl" and "alkoxycarbonyl" are similar to acyl except that the R is aryl or alkoxy, respectively.

As used herein, "PEG" is a polyethylene glycol moiety having the formula  $-(\text{OCH}_2\text{CH}_2)_x$ , where x is that number of subunits required to give an average molecular weight 20 ranging from about 200 to about 8000. As used herein, "PPG" is a polypropylene glycol moiety having the formula  $-(\text{OCH}_2\text{CH}_2\text{CH}_2)_x$ , where x is that number of subunits required to give an average molecular weight ranging from about 200 to about 8000.

25 As used herein, "therapeutically-effective amount" refers to that amount necessary to administer to a host to achieve an anti-tumor effect; to induce differentiation and/or inhibition of proliferation of malignant cancer cells, benign tumor cells or other 30 proliferative cells; to aid in the chemoprevention of cancer; to promote wound healing; to treat a gastrointestinal disorder; to treat a blood disorder or increase the hemoglobin content of blood; to modulate an immune response; to enhance recombinant gene expression; 35 to modulate gene expression; to augment expression of tumor suppressor genes; to enhance insulin expression; to

enhance chloride channel expression, to induce tolerance to an antigen; to treat, prevent or ameliorate protozoan infection; or to inhibit histone deacetylase in cells. Methods of determining therapeutically-effective amounts are well known.

When the therapeutic or effective amount of the compound is for treating, preventing or ameliorating cancer or other proliferative disorder, then that amount can be an amount effective to inhibit histone deacetylase in the subject, patient or cancerous cells. Similarly, when the therapeutic or effective amount of the compound is for treating, preventing, or ameliorating protozoan infection then that amount can be an amount effective to inhibit protozoan histone deacetylase in the subject, patient or cancerous cells.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds that are modified by making acid salts. Examples include, but are not limited to, mineral or organic acid salts of basic residues such as amines. Pharmaceutically acceptable salts include, but are not limited to, hydrohalides, sulfates, methosulfates, methanesulfonates, toluenesulfonates, nitrates, phosphates, maleates, acetates, lactates and the like.

Pharmaceutically-acceptable salts of the compounds of the invention can be prepared by reacting the free base forms of these compounds with a stoichiometric or greater amount of the appropriate acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. The salts of the invention can also be prepared by ion exchange, for example. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the

disclosure of which is hereby incorporated by reference in its entirety.

The "pharmaceutical agents" for use in the methods of the invention related to the coadministration of 5 compounds of Formula II and Formula III, include but are not limited to anticancer agents as well as differentiating agents. For example, the pharmaceutical agent can be a cytokine, an interleukin, an anti-cancer agent, a chemotherapeutic agent, an antibody, a 10 conjugated antibody, an immune stimulant, an antibiotic, a hormone antagonist or a growth stimulant. The pharmaceutical agent can also be a cytotoxic agent. Cytotoxic agents include antiviral nucleoside antibiotics such as ganciclovir, acyclovir, and famciclovir. 15 Cytotoxic agents can also include radiation therapy.

As used herein, the "chemotherapeutic agents" include but are not limited to alkylating agents, purine and pyrimidine analogs, vinca and vinca-like alkaloids, etoposide and etoposide-like drugs, corticosteroids, 20 nitrosoureas, antimetabolites, platinum-based cytotoxic drugs, hormonal antagonists, anti-androgens and antiestrogens.

The "cytokines" for use herein include but are not limited to interferon, preferably  $\alpha$ ,  $\beta$  or  $\gamma$  interferon, 25 as well as IL-2, IL-3, G-CSF, GM-CSF and EPO.

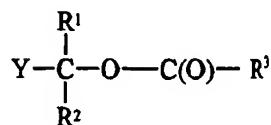
As used herein, an "immune stimulant" is a substance such as *C. parvum* or sarcolectin which stimulates a humoral or cellular component of the immune system.

The chemotherapeutic agents of the invention include 30 but are not limited to tamoxifen, doxorubicin, L-asparaginase, dacarbazine, amascrine, procarbazine, hexamethylmelamine, mitoxantrone and gemcitabine.

#### SYNTHETIC METHODS

35 The compounds of the present invention can generally be prepared by any method known in the art. For example,

the compounds of the invention can be made by reacting the acid RCOOH with a reagent of the formula



5 or by similar reactions between any of the appropriate acids and the appropriate alkyl halides in the presence of a base, where Y is a leaving group such as halogen, methanesulfonate or *p*-toluenesulfonate and R, R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> are as defined herein. The above reagents are readily  
10 prepared according to literature procedures, see for example, Nudelman et al., *J. Med. Chem.* 35:687-694, 1992, and Japanese patent 07033709 (1995). The base can be a trialkylamine, pyridine, an alkali metal carbonate or other suitable base. The reaction can be carried out in  
15 the presence or absence of an inert solvent. Suitable solvents include, for example, acetone, benzene, toluene, tetrahydrofuran, ethyl acetate, acetonitrile, dimethylformamide, dimethyl sulfoxide, chloroform, dioxan or 1,2-dichloroethane.

20 The procedures outlined above can be improved by one skilled in the art by, for instance, changing the temperature, duration, stoichiometry or other parameters of the reactions. Any such changes are intended to fall within the scope of this invention.

25

#### ACTIVITY

30 The activities of the compounds of the invention can be measured using generally-accepted techniques known to those skilled in the art consistent with the activity of interest. For example, the activity of compounds useful as differentiating agents can be measured using standard methodology of the nitro-blue tetrazolium reduction assay

(e.g., Rabizadeh et al., FEBS Lett. 328:225-229, 1993; Chomienne et al., Leuk. Res. 10:631, 1986; and Breitman et al. in Methods for Serum-free Culture of Neuronal and Lymphoid Cells, Alan R. Liss, NY, p. 215-236, 1984 which are hereby incorporated by reference in their entirety) and as described below. This *in vitro* assay has been deemed to be predictive and in fact correlative with *in vivo* efficacy (Castaigne et al., Blood 76:1704-1709, 1990).

Another assay which is predictive of differentiating activity is the morphological examination for the presence of Auer rods and/or specific differentiation cell surface antigens in cells collected from treatment groups, as described in Chomienne et al., (Blood 76:1710-1717, 1990 which is hereby incorporated by reference in its entirety) and as described below.

The compounds of the present invention also have anti-proliferative and anti-tumor activity. The anti-proliferation activity of compounds of the present invention can be determined by methods generally known to those skilled in the art. Generally-accepted assays for measuring viability and anti-proliferative activity are the trypan blue exclusion test and incorporation of tritiated thymidine, also as described by Chomienne, et al., above, which is incorporated herein by reference. Other assays which predict and correlate antitumor activity and *in vivo* efficacy are the human tumor colony forming assay described in Shoemaker et al., Can. Res. 45:2145-2153, 1985, and inhibition of telomerase activity as described by Hiyayama et al., J. Natl. Cancer Inst. 87:895-908, 1995, which are both incorporated herein by reference in their entirety. These assays are described in further detail below.

Cell Cultures

Human promyelocytic leukemia cells (HL-60), human pancreatic carcinoma cells (PaCa-2) and human breast adenocarcinoma cells, pleural effusion cells (MCF-7) can be cultured as follows. Cells are grown in RPMI media with 10% FCS, supplemented with 2 mM glutamine and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator. Alternatively, cells can be grown in any other appropriate growth medium and conditions which supports the growth of the cell line under investigation. Viability can be determined by trypan blue exclusion. Cells are exposed to a test compound, cultures are harvested at various time points following treatment and stained with trypan blue.

15

Cellular Staining to Detect Differentiation

Lipid staining and/or immunochemical staining of casein can be used as a marker for cellular differentiation of breast cancer cells (Bacus et al., Md. Carcin. 3:350-362, 1990). Casein detection can be done by histochemical staining of breast cancer cells using a human antibody to human casein as described by Cheung et al., J. Clin. Invest. 75:1722-1728, which is incorporated by reference in its entirety.

25

Nitro-Blue Tetrazolium (NBT) Assay:

Cell differentiation of myeloid leukemia cells can be evaluated, for example, by NBT reduction activity as follows. Cell cultures are grown in the presence of a test compound for the desired time period. The culture medium is then brought to 0.1% NBT and the cells are stimulated with 400 nM of 12-O-tetradecanoyl-phorbol-13-acetate (TPA). After incubation for 30 min at 37°C, the cells are examined microscopically by scoring at least 200 cells. The capacity for cells to reduce NBT is assessed as the percentage of cells containing

intracellular reduced black formazan deposits and corrected for viability.

Cell Surface Antigen Immunophenotyping

5 Cell surface antigen immunotyping can be conducted using dual-color fluorescence of cells gated according to size. The expression of a panel of antigens from early myeloid (CD33) to late myeloid can be determined as described in Warrell, Jr. et al., New Engl. J. Med.  
10 324:1385-1392, 1992, which is incorporated by reference herein in its entirety.

Apoptosis Evaluation

15 Apoptosis can be evaluated by DNA fragmentation, visible changes in nuclear structure or immunocytochemical analysis of Bcl-2 expression.

20 DNA fragmentation can be monitored by the appearance of a DNA ladder on an agarose gel. For example, cellular DNA is isolated and analyzed by the method of Martin et al., J. Immunol., 145:1859-1867, 1990 which is incorporated by reference herein in its entirety.

25 Changes in nuclear structure can be assessed, for example, by acridine orange staining method of Hare et al., J. Hist. Cyt., 34:215-220, 1986 which is incorporated by reference herein in its entirety.

30 Immunological detection of Bcl-2 can be performed on untreated cells and cells treated with the test compound. HL-60 cells are preferred but other cell lines capable of expressing Bcl-2 can be used. Cytospins are prepared and the cells are fixed with ethanol. Fixed cells are reacted overnight at 4°C with the primary monoclonal antibody, anti-Bcl-2 at a dilution of 1:50. Staining is completed to visualize antibody binding, for example, using Strep A-B Universal Kit (Sigma) in accordance with 35 the manufacturer's instructions. Identically-treated cells which received no primary antibody can serve as a

non-specific binding control. Commercial kits are also available and can be used for detecting apoptosis, for example, Oncor's Apop Tag®.

5 Modulation of Gene Expression

The levels of expression from oncogene and tumor suppressor genes can be evaluated by routine methods known in the art such as Northern blotting of RNA, immunoblotting of protein and PCR amplification.

10

Mouse Cancer Model

15

Compounds can be examined for their ability to increase the life span of animals bearing B16 melanomas, Lewis lung carcinomas and myelomonocytic leukemias as described in Nudelman et al., J. Med. Chem. 35:687-694, 1992, or Rephaeli et al., Int. J. Cancer 49:66-72, 1991, which are incorporated by reference herein in their entireties.

20

For example, the efficacy of compounds of the present invention in a leukemia model can be tested as follows: Balb/c mice are injected with WEHI cells and a test compound or control solution is administered the following day. The life span of the treated animals is compared to that of untreated animals.

25

The efficacy of compounds of the present invention on primary tumors can also be tested with subcutaneously implanted lung carcinoma or B16 melanoma by measuring the mass of the tumor at the site of implantation every two weeks in control and treated animals.

30

The efficacy of compounds in xenografts can be determined by implanting the human tumor cells subcutaneously into athymic mice. Human tumor cell lines which can be used include, but are not limited to, prostate carcinoma (human Pc-3 cells), pancreatic carcinoma (human Mia PaCa cells), colon adenocarcinoma (human HCT-15 cells) and mammary adenocarcinoma (human

MX-1 cells). Treatment with control solution or a test compound of the invention begins, for example, when tumors are approximately 100 mg. Anti-tumor activity is assessed by measuring the delay in tumor growth, and/or tumor shrinking and/or increased survival of the treated animals relative to control animals.

#### Telomerase Activity

High levels of telomerase activity is associated with the high proliferation rate found in cancer cells. Compounds which inhibit telomerase activity results in inhibition of cancer cell growth and de-differentiation. Commercially available telomerase assays can thus be used to assess the anticancer activities of compounds on cancer cell lines.

#### Chemoprevention

The chemoprevention activity of the compounds of the invention can be determined in the two-stage mouse carcinogenesis model of Nishimo *et al.* (*supra*).

#### Assay of Compounds

Compounds of the invention, their salts or metabolites, can be measured in a biological sample by any method known to those skilled in the art of pharmacology, clinical chemistry or the like. Such methods for measuring these compounds are standard methods and include, but are not limited to high performance liquid chromatography (HPLC), gas chromatography (GC), gas chromatography mass spectroscopy (GC-MS), radioimmunoassay (RIA), and others.

#### Dosage and Formulation

The compounds of the present invention can be administered to a mammalian patient to treat cancer or in any other method of the invention which involves treating

a patient by any means that produces contact of the active agent with the agent's site of action in the body of the subject. Mammalian patients include humans and domestic animals. The compounds of the invention can be 5 administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic agents or in a combination of therapeutic agents. The compounds can be administered alone, but are generally administered with a pharmaceutical carrier 10 selected on the basis of the chosen route of administration and standard pharmaceutical practice. The pharmaceutical compositions of the invention may be adapted for oral, parenteral, transdermal, transmucosal, rectal or intranasal administration, and may be in unit 15 dosage form, as is well known to those skilled in the pharmaceutical art. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion techniques.

The appropriate dosage administered in any given 20 case will, of course, vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the age, general health, metabolism, weight of the recipient and other factors which influence 25 response to the compound; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; and the effect desired. A daily dosage of active ingredient can be expected to be about 10 to 10,000 milligrams per meter<sup>2</sup> of body mass (mg/m<sup>2</sup>), with 30 the preferred dose being 50-5,000 mg/m<sup>2</sup> body mass.

Dosage forms (compositions suitable for 35 administration) contain from about 1 mg to about 1 g of active ingredient per unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-95% by weight based on the total weight of the composition.

The active ingredient can be administered orally in solid or semi-solid dosage forms, such as for example hard or soft-gelatin capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, disperse 5 powders or granules, emulsions, and aqueous or oily suspensions. It can also be administered parenterally, in sterile liquid dosage forms. Other dosage forms include transdermal administration via a patch mechanism or ointment.

10 Compositions intended for oral use may be prepared according to any methods known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents, and 15 preserving agents in order to provide a pharmaceutically elegant and palatable preparation.

Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. Such 20 excipients may include, for example, inert diluents, such as calcium phosphate, calcium carbonate, sodium carbonate, sodium phosphate, or lactose; granulating disintegrating agents, for example, maize starch or 25 alginic acid; binding agents, such as starch, gelatin, or acacia; and lubricating agents, for example, magnesium stearate, stearic acids or talc. Compressed tablets may be uncoated or may be sugar coated or film coated by known techniques to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for 30 selective disintegration and adsorption in the gastrointestinal tract.

Hard gelatin capsules or liquid filled soft gelatin capsules contain the active ingredient and inert powdered or liquid carriers, such as, but not limited to calcium 35 carbonate, calcium phosphate, kaolin, lactose, lecithin starch, cellulose derivatives, magnesium stearate,

5 stearic acid, arachis oil, liquid paraffin, olive oil, pharmaceutically-accepted synthetic oils and other diluents suitable for the manufacture of capsules. Both tablets and capsules can be manufactured as sustained release-products to provide for continuous release of medication over a period of hours.

10 Aqueous suspensions contain the active compound in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, e.g., sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth, and gum acacia; dispersing or wetting agents, such as a naturally occurring phosphatide, e.g., lecithin, or condensation 15 products of an alkylene oxide with fatty acids, for example of polyoxyethylene stearate, or a condensation products of ethylene oxide with long chain aliphatic alcohols, e.g., heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial 20 esters derived from fatty acids and a hexitol, e.g., polyoxyethylene sorbitol monooleate, or a condensation product of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, e.g., polyoxyethylene sorbitan monooleate. The aqueous 25 suspensions can also contain one or more preservatives, for example ethyl or *n*-propyl *p*-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin, or sodium or calcium cyclamate.

30 Dispersable powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those 35 already mentioned above. Additional excipients, for

example, sweetening, flavoring, and coloring agents, can also be present.

Syrups and elixirs can be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such 5 formulations may also contain a demulcent, a preservative and flavoring and coloring agents.

The pharmaceutical compositions can be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous suspension. This suspension 10 can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally-acceptable diluent or solvent, for example, as a solution 15 in 1,3-butanediol.

In general, water, a suitable oil, saline, aqueous dextrose (glucose), polysorbate and related sugar 20 solutions, emulsions, such as Intralipid® (Cutter Laboratories, Inc., Berkley CA) and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Antioxidizing agents, such as but not limited to sodium bisulfite, sodium 25 sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used can be citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as but not limited to benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

30 The pharmaceutical compositions of the present invention also include compositions for delivery across cutaneous or mucosal epithelia including transdermal, intranasal, sublingual, buccal, and rectal administration. Such compositions may be part of a 35 transdermal device, patch, topical formulation, gel, etc., with appropriate excipients. Thus, the compounds

of the present invention can be compounded with a penetration-enhancing agent such as 1-n-dodecylazacyclopentan-2-one or the other penetration-enhancing agents disclosed in U.S. Patent Nos. 3,991,203 and 4,122,170 which are hereby incorporated by reference in their entirety to describe penetration-enhancing agents which can be included in the transdermal or intranasal compositions of this invention.

Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in this field, which is incorporated herein by reference in its entirety.

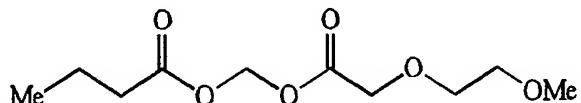
Various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The foregoing disclosure includes all the information deemed essential to enable those skilled in the art to practice the claimed invention. Because the cited patents or publications may provide further useful information these cited materials are hereby incorporated by reference in their entirety.

## EXAMPLE 1

Synthesis of 2- (2-Methoxyethoxy)acetyloxymethyl Butyrate

2- (2-Methoxyethoxy)acetyloxymethyl butyrate  
5 (Compound C) has the following structure



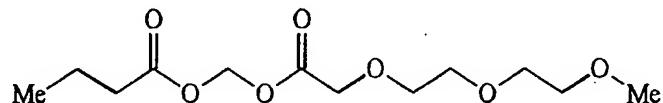
and was synthesized as follows. To a solution of chloromethyl butyrate (682 mg, 5 mmol) in acetone (10 mL), 2-(2-methoxyethoxy)acetic acid (570 mg, 5 mmol) was added, followed by the dropwise addition of triethylamine (0.7 mL, 0.5 g, 1 eq). The mixture was heated at 40°C for 20 h, until TLC (ethyl acetate:hexane 2:1, ninhydrin) indicated the total consumption of the starting materials. The precipitate was filtered and washed with acetone. The filtrate was evaporated and the residue (1.08 g) was chromatographed on silica gel (ethyl acetate:hexane, 2:1) to give the product as an oil, 520 mg (44% yield).

## EXAMPLE 2

Synthesis of2-[2-(Methoxyethoxy)ethoxy]acetyloxymethyl Butyrate  
and Additional Compounds

5

2-[2-(Methoxyethoxy)ethoxy]acetyloxymethyl butyrate (Compound D) has the following structure



10 and was synthesized as follows. To a solution of chloromethyl butyrate (682 mg, 5 mmol) in acetone (10 mL), 2-[2-(methoxyethoxy)ethoxy]acetic acid (890 mg, 5 mmol), was added, followed by the dropwise addition of triethylamine (0.7 mL, 0.5 g, 1 eq). The mixture was  
15 heated at 40°C for 20 h, until TLC (ethyl acetate:hexane 2:1, ninhydrin) indicated complete consumption of the starting materials. The precipitate was filtered and washed with acetone. The filtrate was evaporated and the residue (1.35 g) was chromatographed on silica gel (ethyl acetate:hexane, 2:1), to give the product as an oil (390 mg, 28% yield).

20 Additional compounds of the invention are provided in Table I. These compounds are those of Formula I having the designated groups. These compounds can be  
25 synthesized in a manner analogous to Examples 1 and 2 or as provided in the Detailed Description of the Invention.

TABLE 1  
Additional Compounds

## EXAMPLE 3

Clonogenicity of Established Tumor Cell Lines

Inhibition of tumor growth was tested using the  
5 neuroblastoma cell lines listed in Table 2. Cells were  
grown to 70-80% confluence in complete media [RPMI 1640  
containing 10% FCS, 100 IU penicillin, 100  $\mu$ g/mL  
streptomycin and 2 mM L-glutamine], harvested, washed in  
complete media, and counted. Cell viability was  
10 determined by trypan blue exclusion. The cells were  
placed into soft agar (0.12% in media) and plated at  
5,000 viable cells per well onto an agarose underlayer  
(0.4%) in 24-well plates. After overnight culture, AB or  
Compound C was added at the indicated concentration.  
15 Control cells received media alone. As a control for  
cell death, cells were treated with a superlethal dose of  
10  $\mu$ g/ml of cisplatin. The dosage of AB or Compound C  
which inhibited fifty percent (or ninety percent) of cell  
proliferation ( $IC_{50}$  or  $IC_{90}$ ) was calculated using the Chou  
20 Analysis' Median Effective Dose equation.

Clonogenicity is determined as the percentage of  
clones in treated cultures relative to clones in media-  
treated control cultures. The  $IC_{50}$  and  $IC_{90}$  values of AB  
and Compound C for the neuroblastoma cancer cell lines  
25 are provided in Table 2.

The results demonstrate that Compound C is a more  
potent growth inhibitor than AB. The data show that  
Compound C and AB inhibit cell proliferation in a dose-  
dependent manner but that the cells are at least an order  
30 of magnitude more sensitive to Compound C. The ratio of  
 $IC_{50}$  AB: $IC_{50}$  Compound C ranges between 6.5- to 77-fold with  
a median of 34  $\mu$ M. Similarly the ratio of  $IC_{90}$  AB: $IC_{90}$   
Compound C ranges between 5.8- to 342-fold with a median  
value of 36  $\mu$ M. The  $IC_{90}$  values are clinically important  
35 for assessing eradication of residual cancer disease.

TABLE 2  
 Inhibition of Established Tumor Cell Lines  
 by AB and Compound C

5

10

15

Cell Line	AB		Compound C	
	IC <sub>50</sub> <sup>(a)</sup>	IC <sub>90</sub> <sup>(a)</sup>	IC <sub>50</sub> <sup>(a)</sup>	IC <sub>90</sub> <sup>(a)</sup>
SK-N-SH	998	3397	13	29
NBAS-5	833	13030	19	38
SK-N-MC	215	1314	29	224
IMR-32	881	3566	26	44
NGP	197	1622	30	54
LA1-5S	1627	2675	21	38
SMS-KCN	1872	ND	152	ND
NBL-W-N	489	3074	34	78
SMS-KAN	1138	2079	27	58

(a) All concentrations are in  $\mu$ M.

EXAMPLE 4  
Inhibition of Cancer Cell Proliferation  
Assessed by the SRB Assay

5        The inhibition of cell proliferation was measured in  
the cancer cell lines of Table 3 using the  
sulforhoamine B (SRB) assay as described by Monks et al.,  
1991, J. Natl. Can. Inst. 83:757-766. The SRB assay is  
used to screen for anti-cancer drugs. A representative  
10      clonogenicity titration curve for AB, Compound C and  
Compound D is shown in Fig. 1 for the MCF-7 and CFPAC  
cell lines. The  $IC_{50}$  and  $IC_{90}$  values of AB, Compound C and  
Compound D for each cancer cell line are provided in  
Table 3. A comparison of Compound C, Compound D and AB  
15      demonstrates that Compound C and Compound D at least an  
order of magnitude greater activity than AB as measured  
by the  $IC_{50}$  and  $IC_{90}$  values.

20      TABLE 3  
Human Tumor Cell Lines

Cell Lines	Origin
MCF7	Breast Carcinoma
PC3	Prostate Carcinoma
CFPAC	Pancreatic Carcinoma
HL-60	Promyelocytic Leukemia
HT29	Colon Carcinoma
LOXIMVI	Melanoma
SF-295	Glioblastoma

TABLE 4  
 Comparison of AB, Compound C and  
 Compound D In The SRB Assay

Cell	AB		Compound C		Compound D	
	IC <sub>50</sub>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>
CFPAC	>4.000	>4.000	0.2077	3.6386	0.1853	>4.000
HL-60	1.6438	3.4547	0.2048	0.2480	0.0916	0.7441
HT29	>4.000	>4.000	0.2146	0.4317	0.1637	0.2272
LOX IMVI	>4.000	>4.000	0.1880	0.2402	0.1919	0.2451
MCF7	>4.000	>4.000	0.1560	0.2332	0.0953	0.1224
PC-3	>4.000	>4.000	0.3403	0.4763	0.3694	0.4745
SF-295	>4.000	>4.000	0.1903	0.2425	0.1900	0.2421

15 (a) Expressed in mM

## EXAMPLE 5

Effect Compounds C, D and AN-9 on  
Hyperproliferating Cells

5        The SRB assay was used to compare the effects of Compound C, compound D and AN-9 on the proliferation of hyperproliferating, immortalized human keratinocytes as described in Example 4 (Fig. 2). The IC<sub>50</sub> and IC<sub>90</sub> of Compound C on the keratinocytes was 0.050 and 0.106 mM, 10 respectively, and the IC<sub>50</sub> and IC<sub>90</sub> of Compound D on the keratinocytes was 0.050 and 0.108 mM, respectively. In contrast, the same parameters for AN-9 were 0.108 and 0.221 mM, respectively.

EXAMPLE 6  
Inducing Differentiation

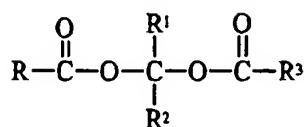
5           Cancer cell differentiation was evaluated in human  
leukemia cell line HL-60 by changes in expression of  
myelocytic maturation marker CD11b.

10           The level of CD11b was measured on HL-60 cells by  
flow cytometry using a monoclonal antibody (MAb) against  
CD11b in a standard indirect immunofluorescence assay.  
15           Cells were cultured for 6 days with the indicated  
concentration of Compound C or AN-9. Cultured cells were  
collected by centrifugation, resuspended at  $10^6$  cells per  
20 $\mu$  RPMI+10% FCS and incubated with MAb (PE-anti-CD11b)  
for 60 min at 4°C. The cells were washed twice in cold  
15           PBS + 10% FCS and incubated with a 1:20 dilution of FITC-  
conjugated F(ab')<sup>2</sup> fragment of rabbit anti-mouse IgG for  
20-30 min at 4°C in the dark. After washing the cells  
twice in cold PBS + 10% FCS, the flow cytometry was  
performed. The results are shown in Fig. 3. Compound C  
20           was a more active differentiation inducer than was AN-9.

WE CLAIM:

1. A compound represented by the formula:

5



wherein

R is C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl or C<sub>2</sub> to C<sub>10</sub> alkynyl, optionally substituted with hydroxy, alkoxy, halo, trifluoromethyl, aryl or heteroaryl;

10 R<sup>1</sup> and R<sup>2</sup> are independently H, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>2</sub>-C<sub>10</sub> alkenyl, or C<sub>2</sub>-C<sub>10</sub> alkynyl, any of which can be optionally substituted with halo, alkoxy, amino, trifluoromethyl, aryl or heteroaryl;

R<sup>3</sup> is

15 (1) C<sub>1</sub>-C<sub>12</sub> alkyl or C<sub>2</sub>-C<sub>10</sub> alkenyl substituted with from one to ten substituents selected from the group consisting of hydroxy, alkoxy, acyloxy, aroyloxy, and aryloxy, and, optionally having one or more halo groups, wherein

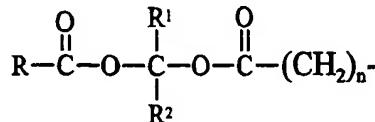
20 adjacent hydroxy groups, if present, can optionally be present as an acetonide,

(2) -(CH<sub>2</sub>)<sub>n</sub>-[O-(CH<sub>2</sub>)<sub>p</sub>]<sub>m</sub>-OR<sup>4</sup>,

(3) -(CH<sub>2</sub>)<sub>n</sub>-PEG-OR<sup>4</sup>, or

(4) -(CH<sub>2</sub>)<sub>n</sub>-PPG-OR<sup>4</sup>;

25 R<sup>4</sup> is C<sub>1</sub> to C<sub>6</sub> alkyl, C<sub>2</sub> to C<sub>6</sub> alkenyl, aryl, acyl, aroyl, alkoxycarbonyl, aminocarbonyl or



PEG is a polyethylene glycol moiety having an average molecular weight of 200-8000;

PPG is a polypropylene glycol moiety having an average molecular weight of 200-8000;

5 p is 2 to 4;

n is 1 to 5;

m is 1 to 6; or

a pharmaceutically-acceptable salt thereof;

10 with the proviso that when R is alkyl, R<sup>3</sup> can not be monohydroxy-n-propyl.

2. The compound of Claim 1, wherein R<sup>3</sup> is -(CH<sub>2</sub>)<sub>n</sub>-[O-(CH<sub>2</sub>)<sub>p</sub>]<sub>m</sub>-OR<sup>4</sup>, R<sup>4</sup> is alkyl, p is 2 or 3, n is 1 or 2, and m is 1, 2 or 3.

15

3. The compound of Claim 2, wherein R<sup>3</sup> is -(CH<sub>2</sub>)<sub>n</sub>-(OCH<sub>2</sub>CH<sub>2</sub>)<sub>m</sub>-OR<sup>4</sup>.

20 4. The compound of Claim 3, wherein said compound is 2-(2-methoxyethoxy)acetyloxymethyl butyrate, 2-[2-(methoxyethoxy)ethoxy]acetyloxymethyl butyrate, 1-[2-(2-methoxyethoxy)acetyl]oxyethyl butyrate, or 1-{2-[2-(2-methoxyethoxy)ethoxy]acetyl}oxyethyl butyrate.

25 5. A pharmaceutical composition comprising a therapeutically-effective amount of a compound of any one of Claims 1 to 4 and a pharmaceutically-effective carrier or diluent.

30 6. A pharmaceutical composition comprising a therapeutically-effective amount of a compound of any one of Claims 1 to 4 with a therapeutically-effective amount of a pharmaceutical agent, wherein said agent is selected from the group consisting of a cytokine, an interleukin, 35 an anti-cancer agent or anti-neoplastic agent, a chemotherapeutic agent, an antibody, a conjugated

antibody, an immune stimulant, an antibiotic, a hormone antagonist or a growth stimulant.

7. The composition of Claim 6 wherein said  
5 pharmaceutical agent comprises a cytotoxic agent.

8. The composition of Claim 6 wherein said  
antibiotic is an antiviral nucleoside antibiotic selected  
from the group consisting of ganciclovir, acyclovir, and  
10 famciclovir.

9. The composition of Claim 6 wherein said  
antibiotic is ganciclovir.

15 10. The composition of Claim 6 wherein said  
chemotherapeutic agent is selected from the group  
consisting of alkylating agents, purine and pyrimidine  
analogs, vinca and vinca-like alkaloids, etoposides and  
etoposide-like drugs, corticosteroids, nitrosoureas,  
20 antimetabolites, platinum based cytotoxic drugs, hormonal  
antagonists, anti-androgens and antiestrogens.

11. The composition of Claim 6 wherein said  
cytokine is an interferon.

25

12. The composition of Claim 6 wherein said immune  
stimulant is *Corynebacterium parvum* or a sarcolectin.

30 13. The composition of Claim 6 wherein the  
chemotherapeutic agent is selected from the group  
consisting of tamoxifen, doxorubicin, L-asparaginase,  
dacarbazine, amascrine, procarbazine, hexamethylmelamine,  
mitoxantrone and gemcitabine.

35

14. A method of treating, preventing or  
ameliorating cancer or other proliferative disorder in a

subject which comprises administering an amount of a compound of any one of Claims 1 to 4 effective to treat, prevent or ameliorate said cancer or said disorder.

5        15. The method of Claim 14 wherein said disorder is leukemia, squamous cell carcinoma, prostate carcinoma, breast carcinoma, colon carcinoma, pancreatic carcinoma, lung carcinoma, renal carcinoma, neuroblastoma or melanoma.

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16. The method of Claim 14 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

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17. The method of Claim 14 wherein said effective amount is an amount effective to inhibit histone deacetylase in said subject.

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18. A method of differentiating or blocking proliferation of cancerous or neoplastic cells comprising administering to said cells an amount of a compound of any one of Claims 1 to 4 effective to cause differentiation of or to block proliferation of cancerous or neoplastic cells.

25

19. The method of Claim 18 wherein said cells are *in vivo*.

30

20. The method of Claim 18 wherein said cells are *in vitro*.

35

21. The method of Claim 18 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

22. A method of increasing hemoglobin content in blood which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 4 to a subject.

5

23. The method of Claim 22 wherein said hemoglobin is fetal hemoglobin.

10 24. The method of Claim 22 wherein said composition is administered orally or parenterally.

15 25. A method of treating a blood disorder in a subject which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 4 to a subject.

20 26. The method of Claim 25, wherein treating said blood disorder comprises increasing the hemoglobin content in cells of said subject.

25

27. The method of Claim 25 wherein said disorder is selected from the group consisting of thalassemias, sickle cell anemias, infectious anemias, aplastic anemias, hypoplastic and hypoproliferative anemias, sideroblastic anemias, myelophthisic anemias, antibody-mediated anemias, anemias due to chronic diseases and enzyme-deficiencies, and anemias due to blood loss, radiation therapy and chemotherapy.

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28. The method of Claim 25 wherein said composition is administered orally or parenterally.

35

29. A method of modulating an immune response in a host which comprises administering an amount of a compound of any one of Claims 1 to 4 effective to modulate said immune response.

30. The method of Claim 29 wherein modulating said immune response is enhancing cytokine secretion, inhibiting or delaying apoptosis in polymorphonuclear cells, enhancing polymorphonuclear cell function by 5 augmenting hematopoietic growth factor secretion, inducing expression of cell surface antigens in tumor cells, enhancing progenitor cell recovery after bone marrow transplantation.

10 31. The method of Claim 29 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

15 32. A method of treating, preventing or ameliorating cancer or other proliferative disorder in a subject in need of such treatment which comprises administering an amount of a compound of any one of 20 Claims 1 to 4 effective to induce cellular apoptosis of the cancer cells or of the cells of the proliferative disorder.

25 33. The method of Claim 32 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

34. A method for enhancing the actions of a pharmaceutical agent useful for the treatment of cancer and other proliferative disorders, comprising co-administering a therapeutically-effective amount of a 30 compound of any one of Claims 1 to 4 with a therapeutically-effective amount of said agent, wherein said agent is selected from the group consisting of a cytokine, an interleukin, an anti-cancer agent or an anti-neoplastic agent, a chemotherapeutic agent, an antibody, a conjugated antibody, an immune stimulant, an 35 antibiotic, a hormone antagonist or a growth stimulant.

35. The method of Claim 34, wherein said antibiotic selected from the group consisting of ganciclovir, acyclovir, and famciclovir.

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36. The method of Claim 34, wherein said chemotherapeutic agent is selected from the group consisting of an alkylating agent, a purine analog, a pyrimidine analog, a vinca alkaloid, a vinca-like alkaloid, etoposide, an etoposide-like drug, a corticosteroid, a nitrosourea, an antimetabolite, a platinum-based cytotoxic drug, a hormonal antagonist, an anti-androgen and an anti-estrogen.

15

37. The method of Claim 34, wherein said cytokine is an interferon.

38. The method of Claim 34, wherein said immune stimulant is *Corynebacterium parvum* or a sarcolectin.

20

39. The method of Claim 34, wherein the chemotherapeutic agent is selected from the group consisting of tamoxifen, doxorubicin, L-asparaginase, dacarbazine, amascrine, procarbazine, hexamethylmelamine, mitoxantrone and gemcitabine.

40. The method of Claim 34, wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

25

41. A method of ameliorating the effects of a cytotoxic agent which comprises administering a therapeutically-effective amount of said cytotoxic agent with a compound of any one of Claims 1 to 4 to a mammalian patient for a time and in an amount to induce growth arrest of rapidly-proliferating epithelial cells

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or bone marrow stem cells of said patient and thereby protecting said cells from cytotoxic effects of said agent.

5       42. The method of Claim 41, wherein said rapidly-proliferating epithelial cells are in hair follicles, gastrointestinal tract or bladder.

10      43. The method of Claim 41, wherein said rapidly-proliferating epithelial cells are hair follicle cells, or intestinal cryt cells.

15      44. The method of Claim 41 wherein administering is simultaneous.

20      45. The method of Claim 41 wherein administering comprises admininstering said agent before or after said compound.

25      46. The method of Claim 41 wherein said administration is systemic or topical.

20      47. A method of treating gastrointestinal disorders which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 4 to a subject.

30      48. The method of Claim 47 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

35      49. A method of treating cutaneous ulcers which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 4 to a subject.

50. The method of Claim 49 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

5 51. A method of inducing wound healing which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 4 to a subject.

10 52. The method of Claim 51 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intrasally, rectally or topically.

15 53. A method of enhancing recombinant gene expression which comprises treating a recombinant host cell containing an expression system for a gene product of interest with an expression-enhancing amount of a compound of any one of Claims 1 to 4, wherein said gene product is encoded by a butyric acid-responsive gene.

20 54. The method of Claim 53, wherein said host cell is a mammalian cell, an insect cell, a yeast cell or a bacterial cell.

25 55. The method of Claim 53, wherein said gene product is a tumor suppressor gene or fetal hemoglobin.

30 56. A method of treating, preventing or ameliorating symptoms in insulin-dependent patients which comprises administering an amount of a compound of any one of Claims 1 to 4 effective to enhance insulin expression.

35 57. The method of Claim 56 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

5 58. A method of treating, preventing or ameliorating symptoms in cystic fibrosis patients which comprises administering an amount of a compound of any one of Claims 1 to 4 effective to enhance chloride channel expression.

10 59. The method of Claim 58 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

15 60. A method of inhibiting telomerase activity in cancer cells which comprises administering to said cells an amount of a compound of any one of Claims 1 to 4 effective to decrease the basal level telomerase activity is said cells and thereby inhibit malignant progression of said cells.

20 61. The method of Claim 60 wherein said cells are *in vivo*.

25 62. The method of Claim 61 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

30 63. The method of Claim 60 wherein said cells are *in vitro*.

35 64. A method of treating, preventing or ameliorating virus-associated tumors which comprises co-administering a therapeutically-effective amount of a compound of any one of Claims 1 to 4 with a therapeutically-effective amount of an antiviral agent.

65. The method of Claim 64 wherein said antiviral agent is ganciclovir, acyclovir, or famciclovir.

66. The method of Claim 64 wherein said virus-associated tumor is an EBV-associated malignancy, Kaposi's sarcoma, an AIDS-related lymphoma, an hepatitis B-associated malignancy or an hepatitis C-associated malignancy.

67. The method of Claim 66 wherein said EBV-associated malignancy is nasopharyngeal carcinoma or non-Hodgkin's lymphoma.

68. The method of Claim 64 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

69. A method of modulating gene expression which comprises treating a host or host cells with an amount of a compound of any one of Claims 1 to 4 effective to enhance, augment or repress expression of a gene of interest.

70. The Method of Claim 69 wherein expression of said gene is enhanced or augmented.

71. The method of Claim 70 wherein said gene encodes a gene product which is or acts as an repressor, a tumor suppressor, an inducer of apoptosis or an inducer of differentiation.

72. The method of Claim 71 wherein said host is a cancer patient and said gene is a tumor suppressor gene.

73. The method of Claim 72 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

74. The method of Claim 69 wherein expression of said gene is repressed.

5 75. The method of Claim 74 wherein said gene encodes a gene product which is or acts as an oncogene or an inhibitor of apoptosis.

10 76. The method of Claim 75 wherein said gene is bcl-2.

77. The method of Claim 69 wherein said host cells are undergoing ex vivo gene therapy.

15 78. The method of Claim 69 wherein said host is a patient receiving in vivo gene therapy.

20 79. The method of Claim 78 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

80. A method of inducing tolerance to an antigen which comprises administering a therapeutically-effective amount of compound of any one of Claims 1 to 4.

25 81. The method of Claim 80 wherein said antigen is a self-antigen.

82. The method of Claim 81 wherein said self-antigen is associated with an autoimmune disease.

30 83. The method of Claim 82 wherein said autoimmune disease is systemic lupus erythematosis, rheumatoid arthritis, multiple sclerosis, myasthenia gravis or diabetes.

84. The method of Claim 80 wherein said antigen is present on a transplanted organ or cells.

5 85. The method of Claim 80 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

10 86. A method for treating, preventing, or ameliorating protozoan infection in a subject which comprises administering to said subject an effective amount of a compound of any one of Claims 1 to 4.

15 87. The method of Claim 86, wherein said protozoan infection is malaria, cryptosporidiosis, toxoplasmosis, or coccidiosis.

20 88. The method of Claim 86, wherein said compound is administered orally, parenterally, transmucosally, intranasally or rectally.

89. The method of Claim 86, wherein said effective amount is an amount of said compound effective to inhibit protozoan histone deacetylase in said subject.

25 90. A method of inhibiting histone deacetylase in cells which comprises administering an effective amount of a compound of any one of Claims 1 to 4 to said cells.

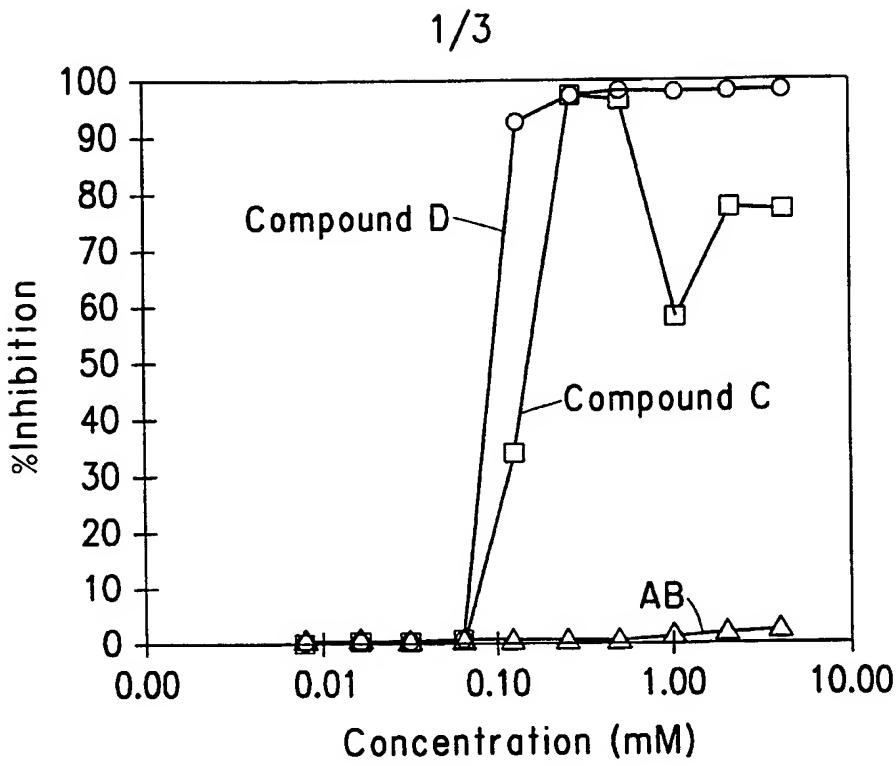


FIG. 1A

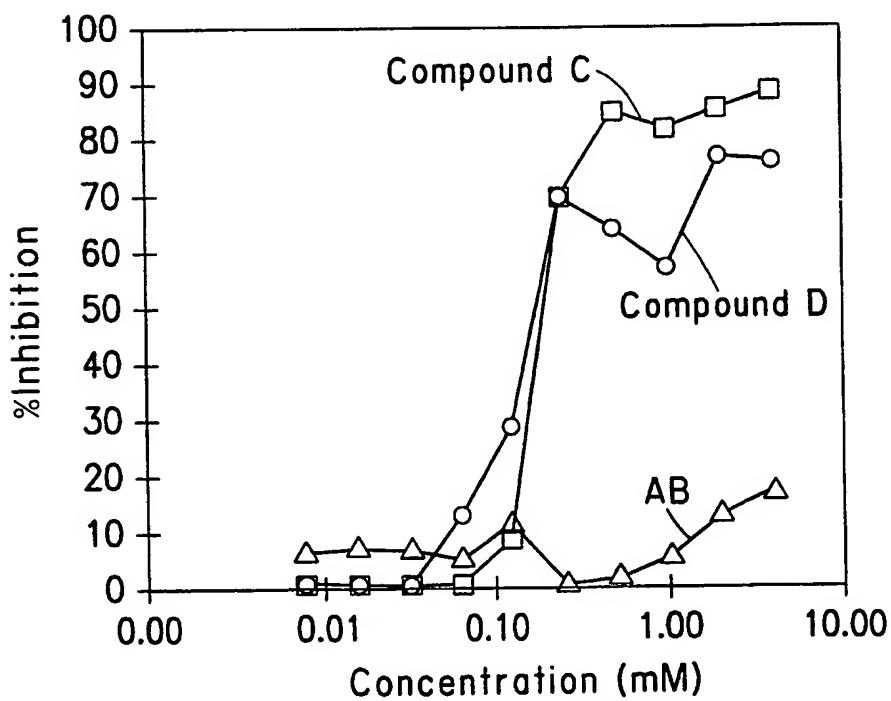


FIG. 1B

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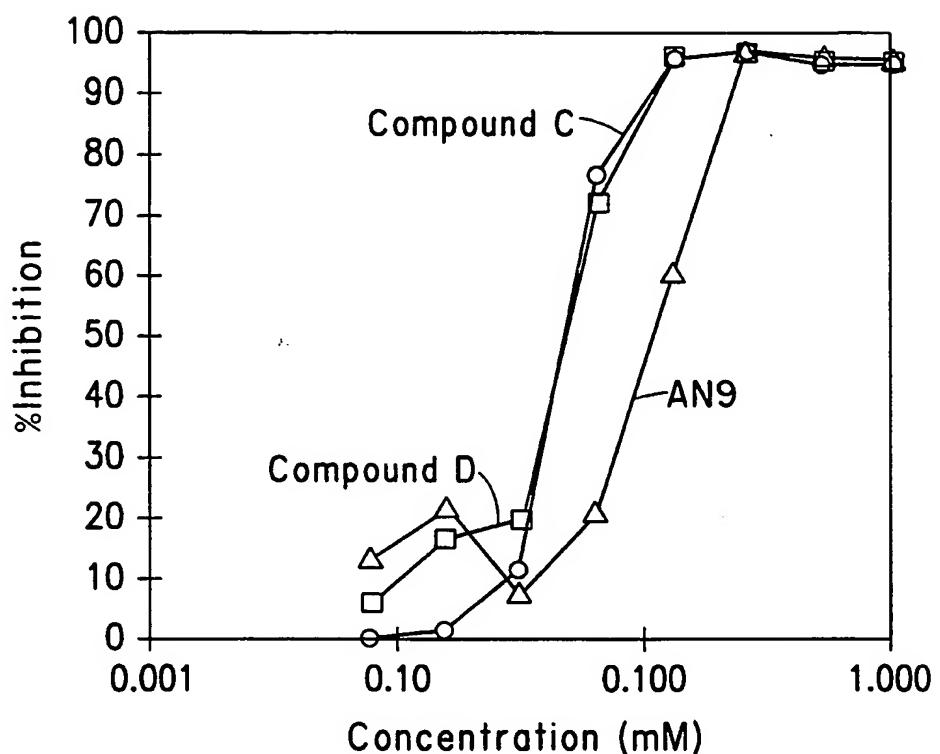


FIG. 2

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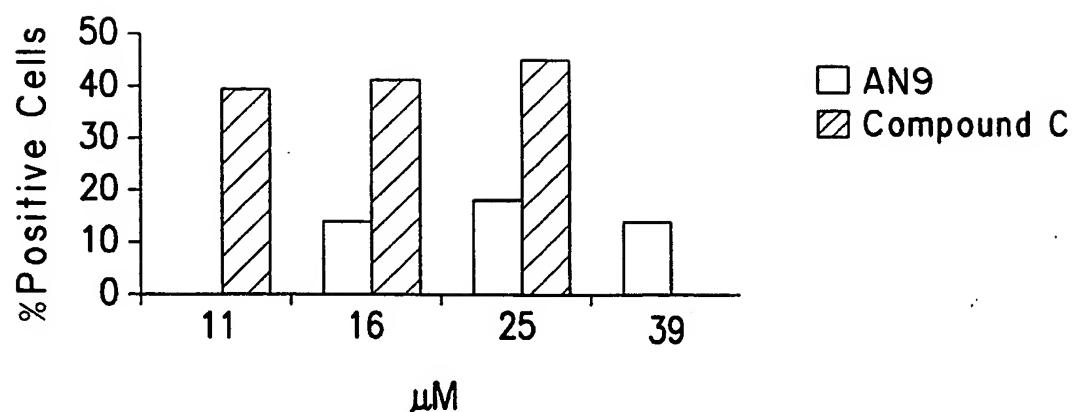


FIG. 3

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/04764

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/22, 235; C07C 69/02, 612  
US CL : 560/186, 187, 61; 549/500, 501, 430

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 560/186, 187, 61; 549/500, 501, 430

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CHEMICAL ABSTRACTS

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 3,700,722 A (MCTEER) 24 October 1972, see entire document.	1-90
A	US 4,479,963 A (GRUENFELD) 30 October 1984, see entire document.	1-90
A	US 4,927,966 A (KALMAN) 22 May 1990, see entire document.	1-90
A	US 5,030,654 A (BARNISH et al) 09 July 1991, see entire document.	1-90
A	US 5,412,137 A (PRASHAD et al) 02 May 1995, see entire document.	1-90

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

10 JUNE 1998

Date of mailing of the international search report

16 JUL 1998

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